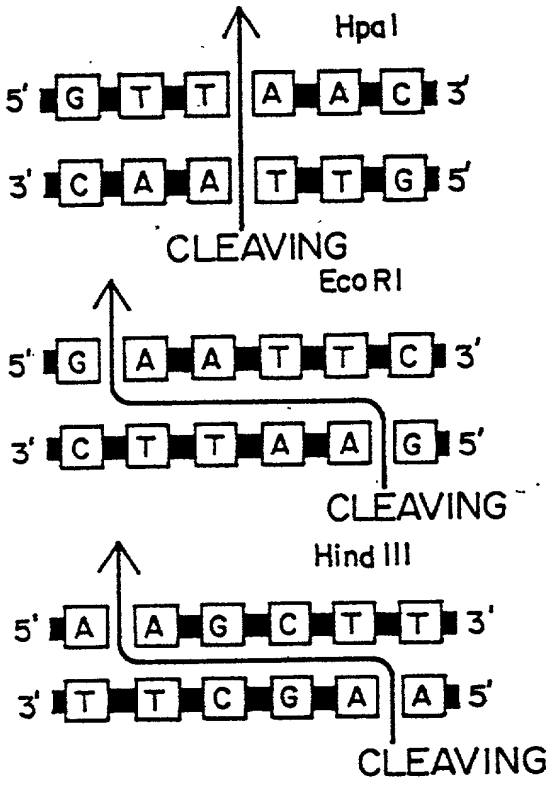

Hpa I
5' G T T A A C 3'
3' C A A T T G 5'
CLEAVING
Eco RI
5' G A A T T C 3'
3' C T T A A G 5'
CLEAVING
Hind III
5' A A G C T T 3'
3' T T C G A A 5'
CLEAVING

FIG. 1

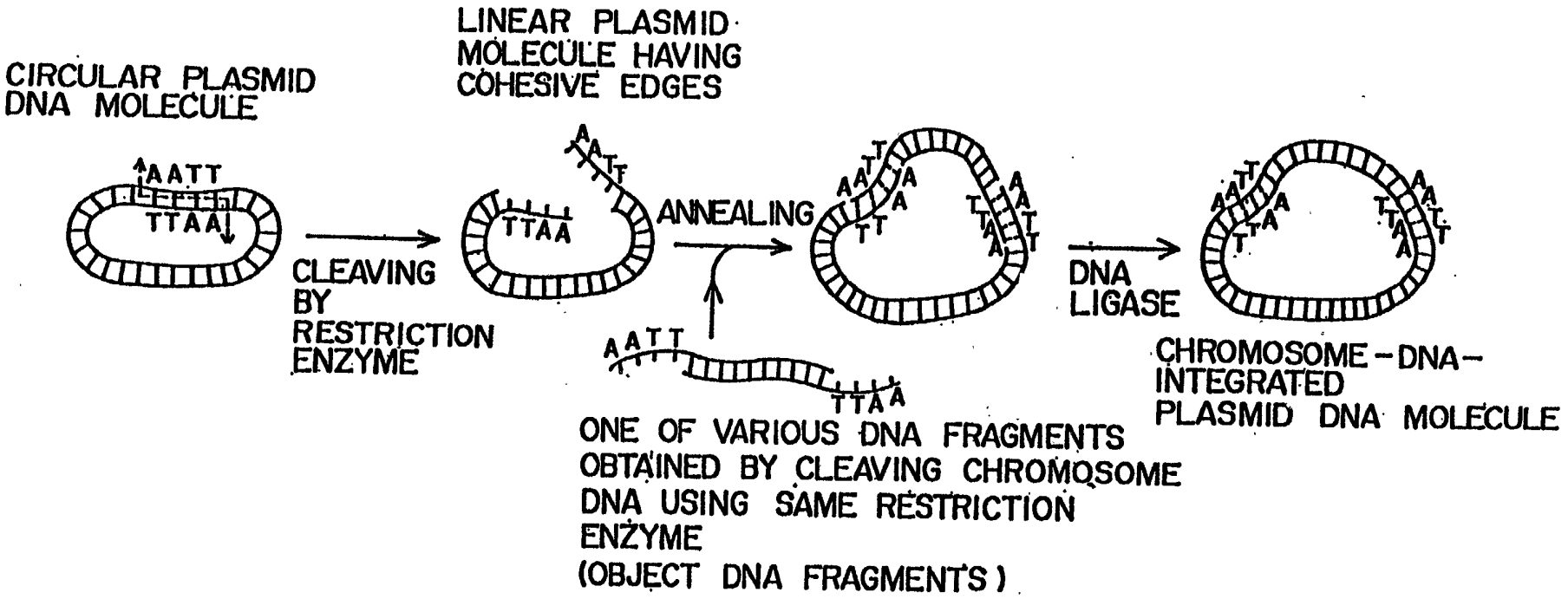


FIG. 2

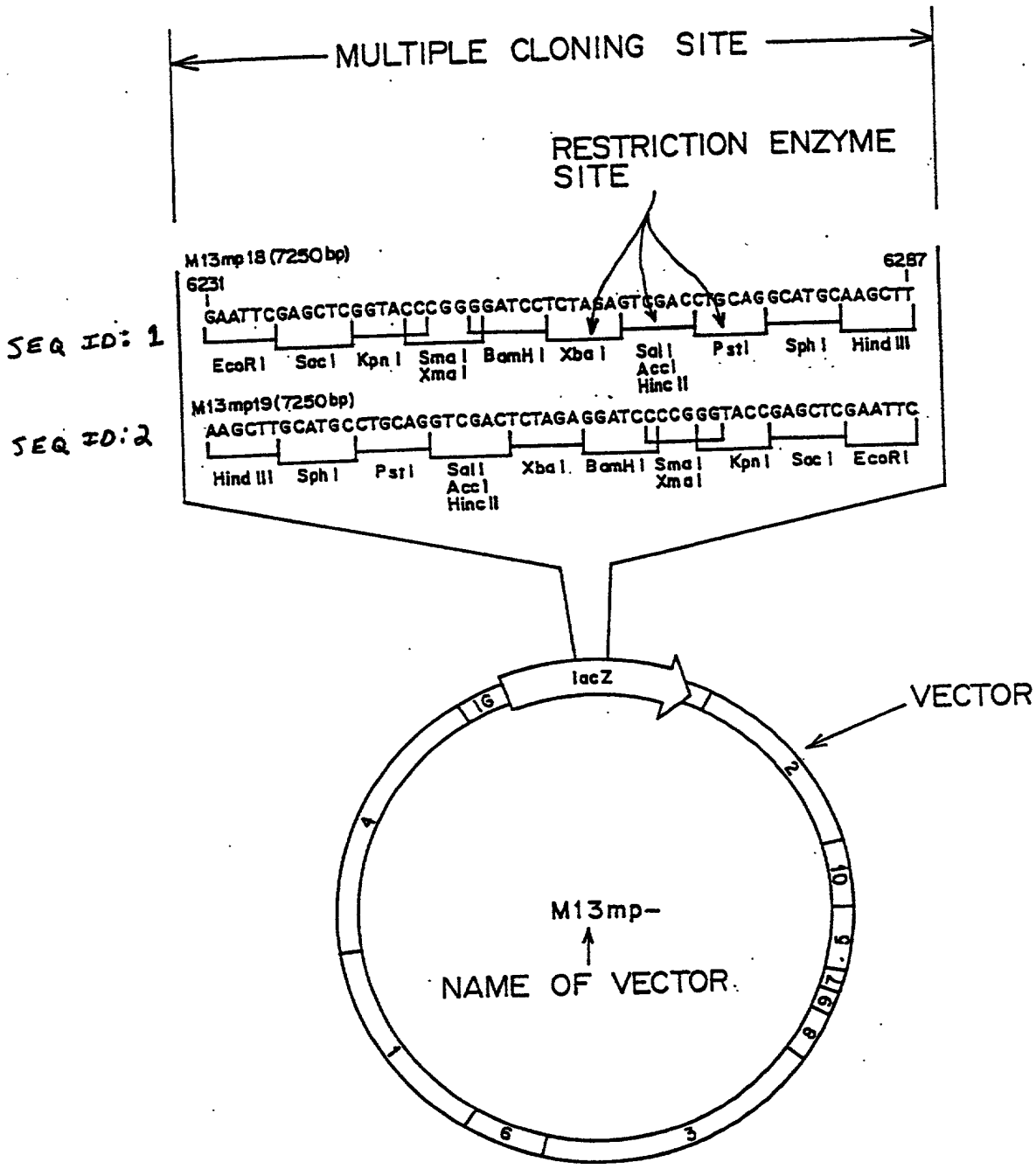

MULTIPLE CLONING SITE
RESTRICTION ENZYME SITE
M13mp18 (7250 bp)
6231
6287
GAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTT
EcoR I
Sac I
Kpn I
Sma I
Xma I
BamH I
Xba I
Sal I
Acc I
Hinc II
Pst I
Sph I
Hind III
SEQ ID: 1
M13mp19 (7250 bp)
AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTC
Hind III
Sph I
Pst I
Sal I
Acc I
Hinc II
Xba I
BamH I
Sma I
Xma I
Kpn I
Sac I
EcoR I
SEQ ID: 2
lacZ
lG
VECTOR
M13mp-
NAME OF VECTOR

FIG. 3

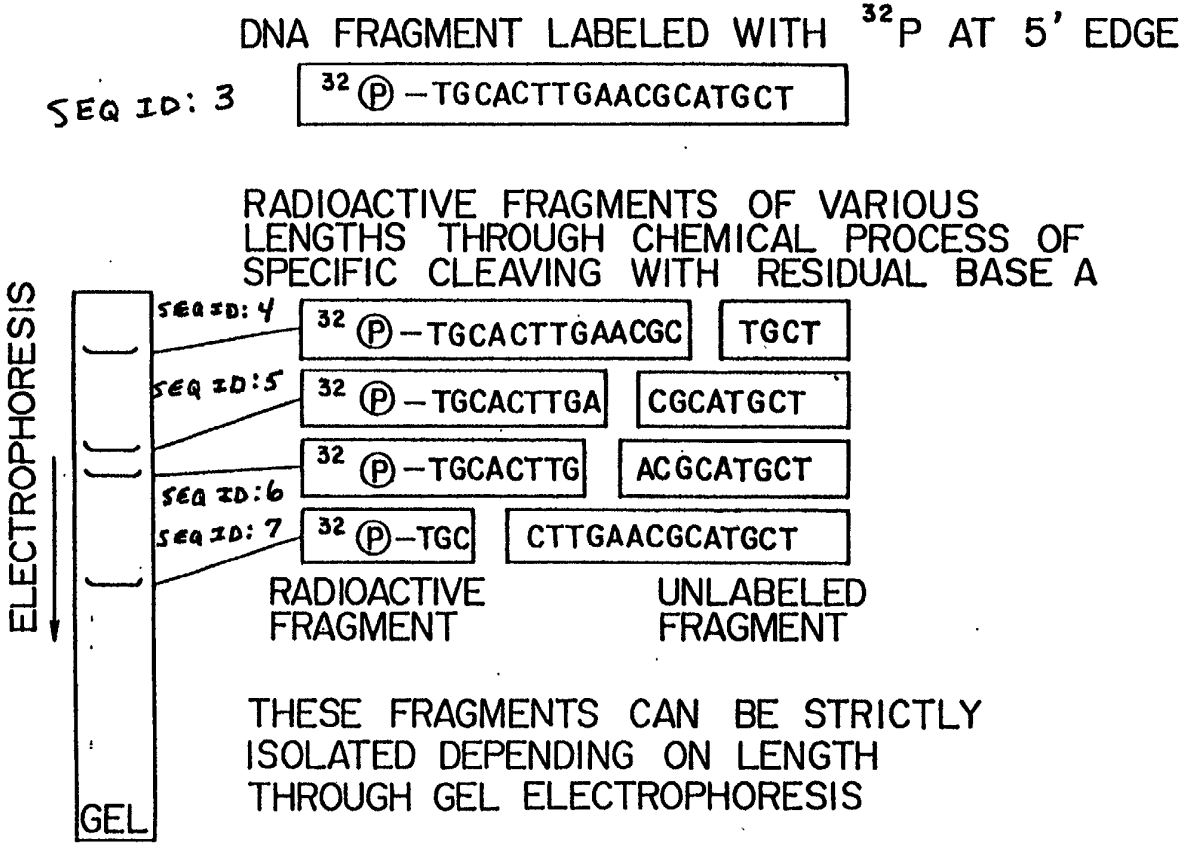
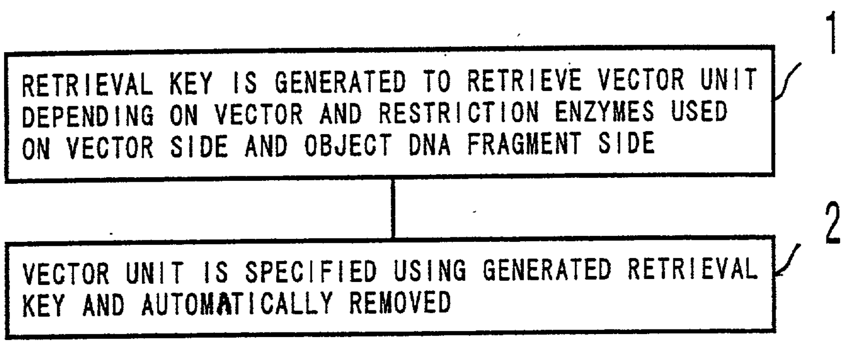

DNA FRAGMENT LABELED WITH ^{32}P AT 5' EDGE
SEQ ID: 3
^{32}Ⓟ–TGCACTTGAACGCATGCT
RADIOACTIVE FRAGMENTS OF VARIOUS LENGTHS THROUGH CHEMICAL PROCESS OF SPECIFIC CLEAVING WITH RESIDUAL BASE A
ELECTROPHORESIS
SEQ ID: 4
^{32}Ⓟ–TGCACTTGAACGC
TGCT
SEQ ID: 5
^{32}Ⓟ–TGCACTTGA
CGCATGCT
SEQ ID: 6
^{32}Ⓟ–TGCACTTG
ACGCATGCT
SEQ ID: 7
^{32}Ⓟ–TGC
CTTGAACGCATGCT
RADIOACTIVE FRAGMENT
UNLABELED FRAGMENT
GEL
THESE FRAGMENTS CAN BE STRICTLY ISOLATED DEPENDING ON LENGTH THROUGH GEL ELECTROPHORESIS

FIG. 4



F I G. 5

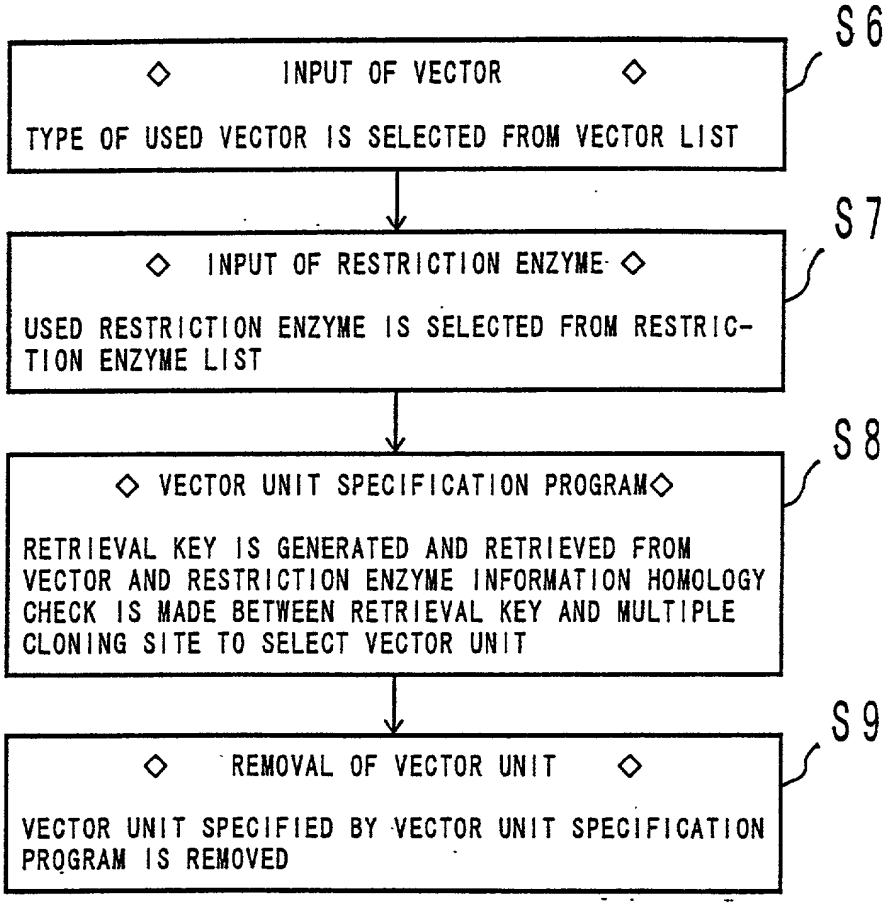


FIG. 6

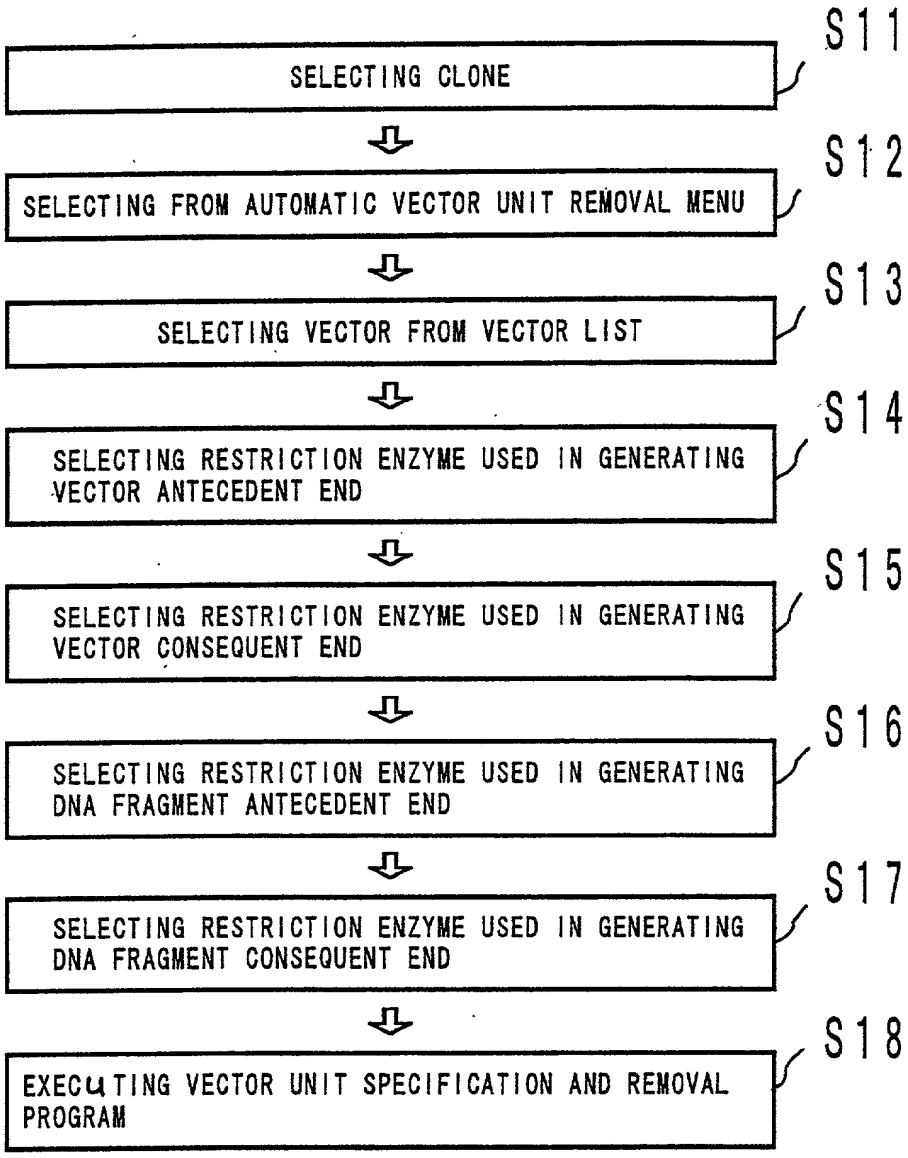

S11
SELECTING CLONE
S12
SELECTING FROM AUTOMATIC VECTOR UNIT REMOVAL MENU
S13
SELECTING VECTOR FROM VECTOR LIST
S14
SELECTING RESTRICTION ENZYME USED IN GENERATING VECTOR ANTECEDENT END
S15
SELECTING RESTRICTION ENZYME USED IN GENERATING VECTOR CONSEQUENT END
S16
SELECTING RESTRICTION ENZYME USED IN GENERATING DNA FRAGMENT ANTECEDENT END
S17
SELECTING RESTRICTION ENZYME USED IN GENERATING DNA FRAGMENT CONSEQUENT END
S18
EXECUTING VECTOR UNIT SPECIFICATION AND REMOVAL PROGRAM

FIG. 7

M13MP18
M13MP19
PBR322
PSL1180
PSL1190
PT7T318U
PT7T319U
PTZ18R
PTZ19R
PUC18
PUC19, ETC.

FIG. 8

VECTOR DB FORMAT

```
>ID
PUC18
>SEQ ID: 8
TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGAT
GCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGA
GCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCC
ATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGG
GGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCAA
GCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCT
GTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATG
AGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAAT
GAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCG
GTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGG
AAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCC
GCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCG
TTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTC
GGGAAGCGTGGCGCTTTCTCAAAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTG
TGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGAC
TTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTG
GTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAG
TTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGA
AAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGAT
TTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTA
TATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCA
TCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGAT
ACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTC
CTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTG
CGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCA
ACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAA
GTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGC
TTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTC
AATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCT
CAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTC
ACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAAT
ACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTA
TTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATC
ATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC
>MULTI
399..450
```

FIG. 9

(* INDICATES MULTIPLE CLONING SITE)

****

SEQ ID: 9 GTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAAT

SEQ ID: 10 AAGCTT⇨HIND III

SEQ ID: 11 GCATGC⇨SPH I

SEQ ID: 12 CTGCAG⇨PST I

SEQ ID: 13 GTCGAC⇨SAL I, ACC I, HINC II

SEQ ID: 14 TCTAGA⇨XBA I

SEQ ID: 15 GGATCC⇨BAMH I

SEQ ID: 16 CCCGGG ⇨SMA I, XMA I

SEQ ID: 17 GGTACC ⇨KPN I

SEQ ID: 18 GAGCTC ⇨SAC I

SEQ ID: 19 GAATTC ⇨ECOR I

FIG. 10

VECTOR SIDE	OBJECT DNA FRAGMENT SIDE
HIND III	HINDIII
SPH I	SPH I
PST I	PST I
SAL I	SAL I
ACC I	ACC I
HINC II	HINC II
XBA I	XBA I
BAMH I	BAMH I
SMA I	SMA I
XMA I	XMA I
KPN I	KPN I
SAC I	SAC I
ECOR I	ECOR I
OTHER RESTRICTION ENZYME	
• • •	

FIG. 11

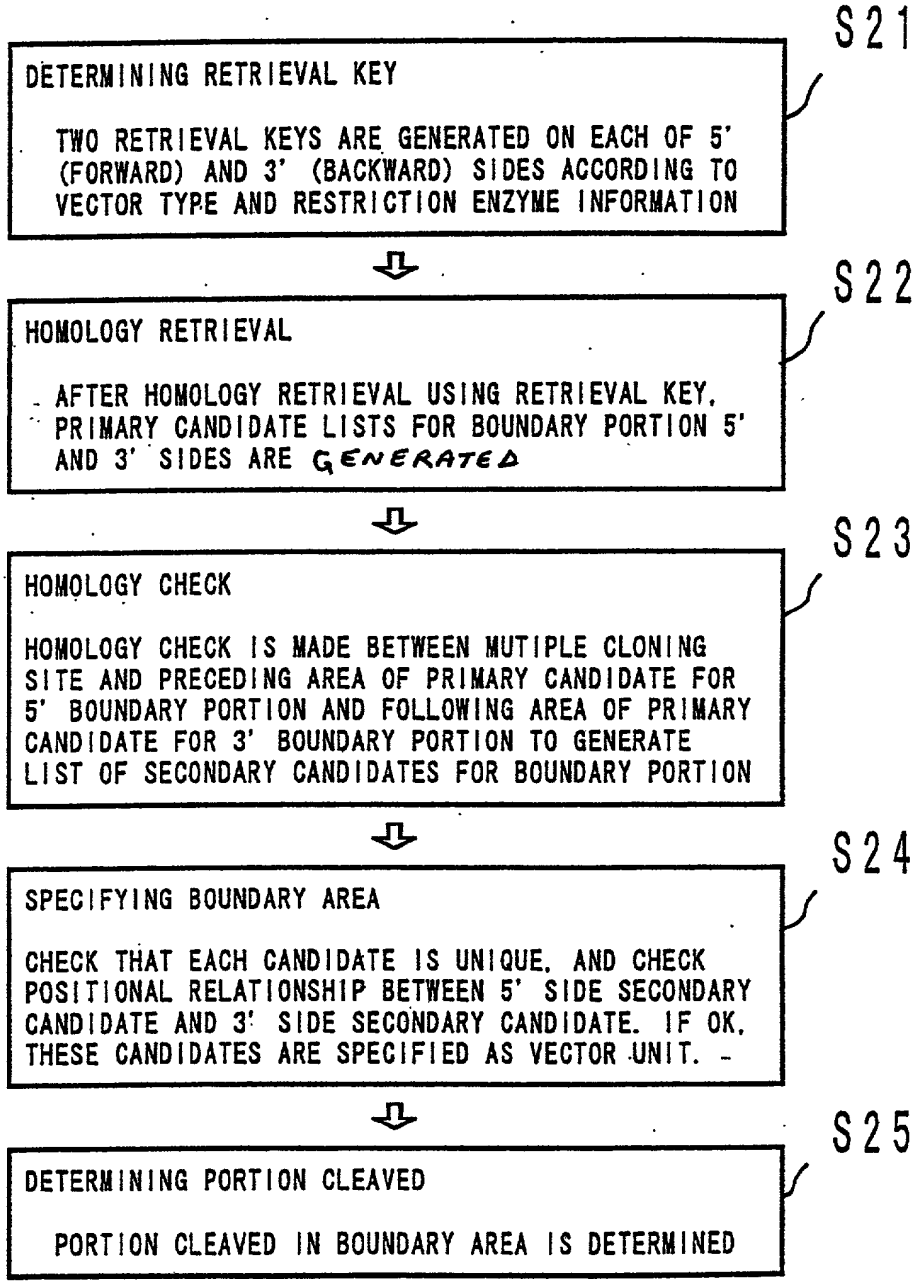

S21
DETERMINING RETRIEVAL KEY
TWO RETRIEVAL KEYS ARE GENERATED ON EACH OF 5' (FORWARD) AND 3' (BACKWARD) SIDES ACCORDING TO VECTOR TYPE AND RESTRICTION ENZYME INFORMATION
S22
HOMOLOGY RETRIEVAL
AFTER HOMOLOGY RETRIEVAL USING RETRIEVAL KEY, PRIMARY CANDIDATE LISTS FOR BOUNDARY PORTION 5' AND 3' SIDES ARE GENERATED
S23
HOMOLOGY CHECK
HOMOLOGY CHECK IS MADE BETWEEN MUTIPLE CLONING SITE AND PRECEDING AREA OF PRIMARY CANDIDATE FOR 5' BOUNDARY PORTION AND FOLLOWING AREA OF PRIMARY CANDIDATE FOR 3' BOUNDARY PORTION TO GENERATE LIST OF SECONDARY CANDIDATES FOR BOUNDARY PORTION
S24
SPECIFYING BOUNDARY AREA
CHECK THAT EACH CANDIDATE IS UNIQUE, AND CHECK POSITIONAL RELATIONSHIP BETWEEN 5' SIDE SECONDARY CANDIDATE AND 3' SIDE SECONDARY CANDIDATE. IF OK, THESE CANDIDATES ARE SPECIFIED AS VECTOR UNIT.
S25
DETERMINING PORTION CLEAVED
PORTION CLEAVED IN BOUNDARY AREA IS DETERMINED

FIG. 12

WHEN SINGLE-STRANDED AREA IS FOUND ON 3' SIDE

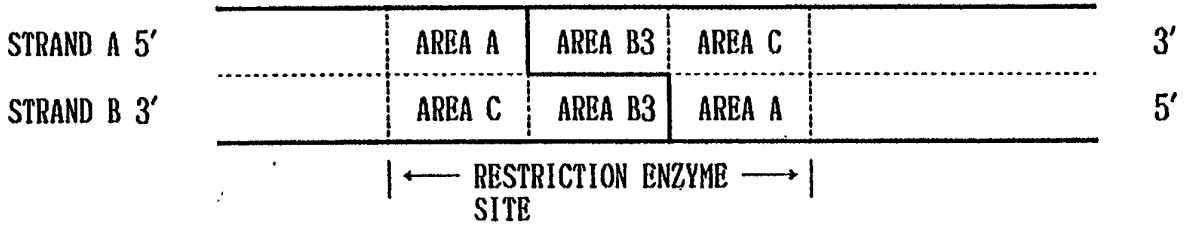


FIG. 13A

WHEN NO SINGLE-STRANDED AREA IS FOUND

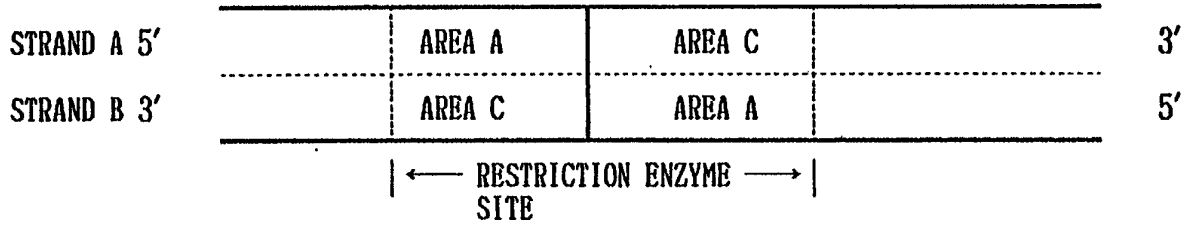


FIG. 13B

WHEN SINGLE-STRANDED AREA IS FOUND ON 5' SIDE

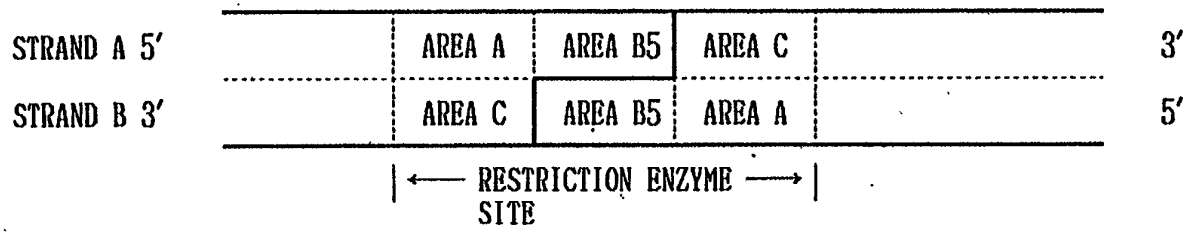


FIG. 13C

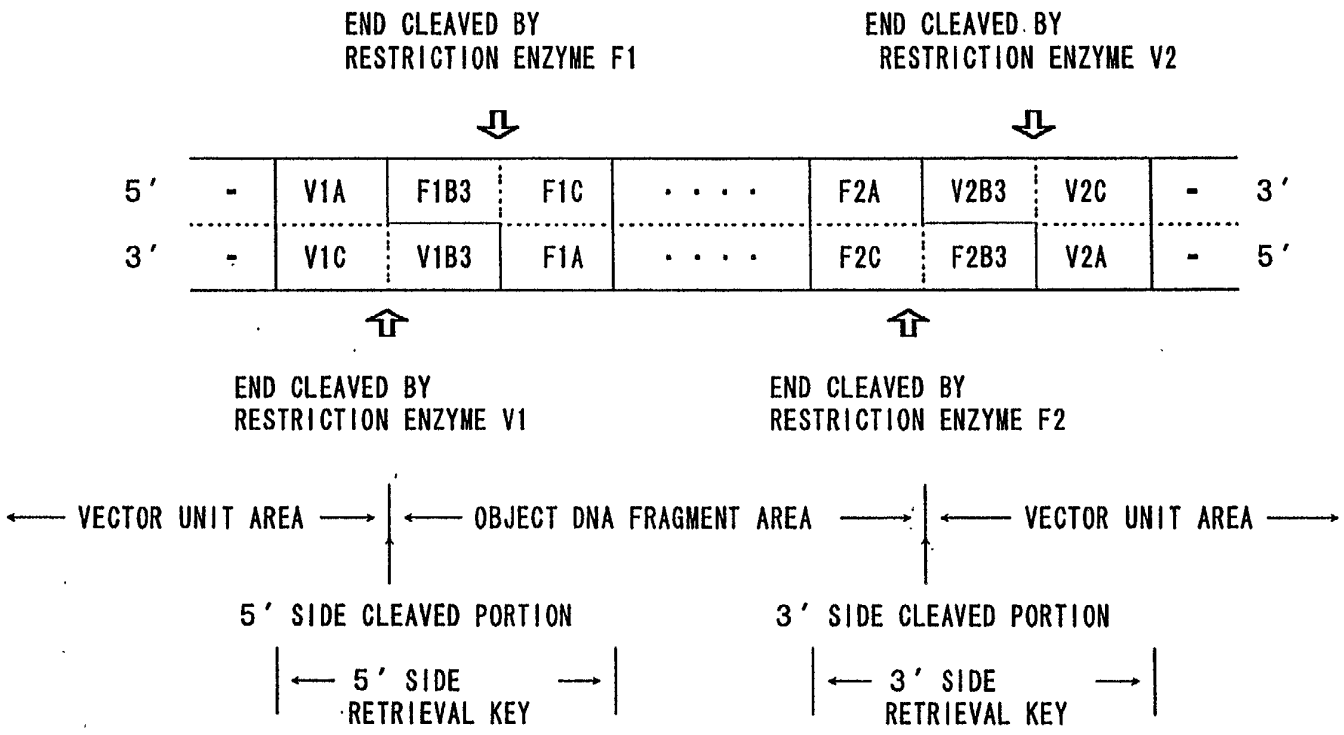

END CLEAVED BY
RESTRICTION ENZYME F1
END CLEAVED BY
RESTRICTION ENZYME V2
5′
3′
-
V1A
F1B3
F1C
· · · ·
F2A
V2B3
V2C
-
3′
-
V1C
V1B3
F1A
· · · ·
F2C
F2B3
V2A
-
5′
END CLEAVED BY
RESTRICTION ENZYME V1
END CLEAVED BY
RESTRICTION ENZYME F2
VECTOR UNIT AREA
OBJECT DNA FRAGMENT AREA
VECTOR UNIT AREA
5′ SIDE CLEAVED PORTION
3′ SIDE CLEAVED PORTION
5′ SIDE
RETRIEVAL KEY
3′ SIDE
RETRIEVAL KEY

FIG. 14

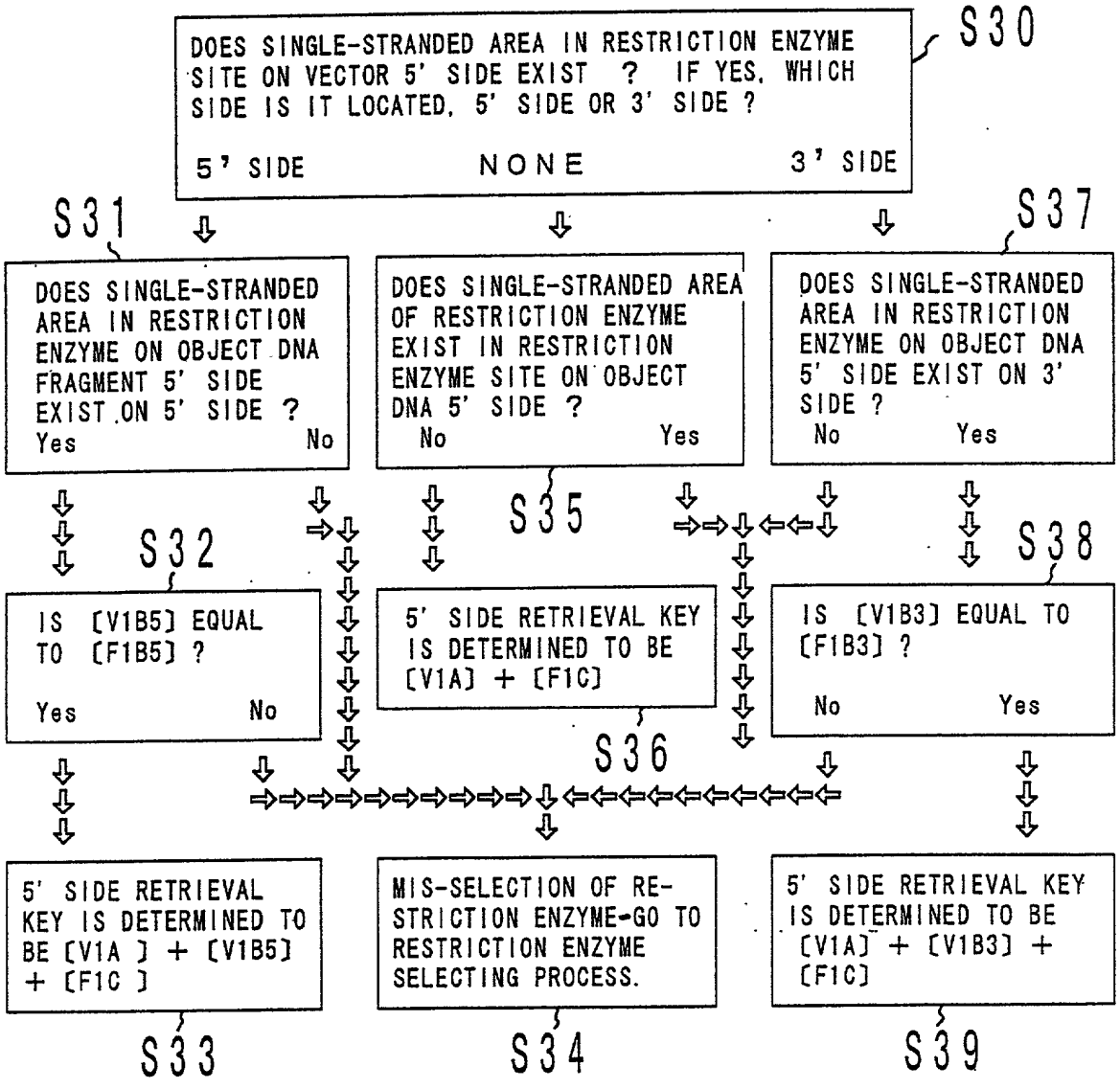

DOES SINGLE-STRANDED AREA IN RESTRICTION ENZYME SITE ON VECTOR 5' SIDE EXIST ? IF YES, WHICH SIDE IS IT LOCATED, 5' SIDE OR 3' SIDE ?
5' SIDE
NONE
3' SIDE
S30
S31
DOES SINGLE-STRANDED AREA IN RESTRICTION ENZYME ON OBJECT DNA FRAGMENT 5' SIDE EXIST ON 5' SIDE ?
Yes
No
S32
IS [V1B5] EQUAL TO [F1B5] ?
Yes
No
5' SIDE RETRIEVAL KEY IS DETERMINED TO BE [V1A] + [V1B5] + [F1C]
S33
DOES SINGLE-STRANDED AREA OF RESTRICTION ENZYME EXIST IN RESTRICTION ENZYME SITE ON OBJECT DNA 5' SIDE ?
No
Yes
S35
5' SIDE RETRIEVAL KEY IS DETERMINED TO BE [V1A] + [F1C]
S36
MIS-SELECTION OF RESTRICTION ENZYME-GO TO RESTRICTION ENZYME SELECTING PROCESS.
S34
S37
DOES SINGLE-STRANDED AREA IN RESTRICTION ENZYME ON OBJECT DNA 5' SIDE EXIST ON 3' SIDE ?
No
Yes
S38
IS [V1B3] EQUAL TO [F1B3] ?
No
Yes
5' SIDE RETRIEVAL KEY IS DETERMINED TO BE [V1A] + [V1B3] + [F1C]
S39

FIG. 15

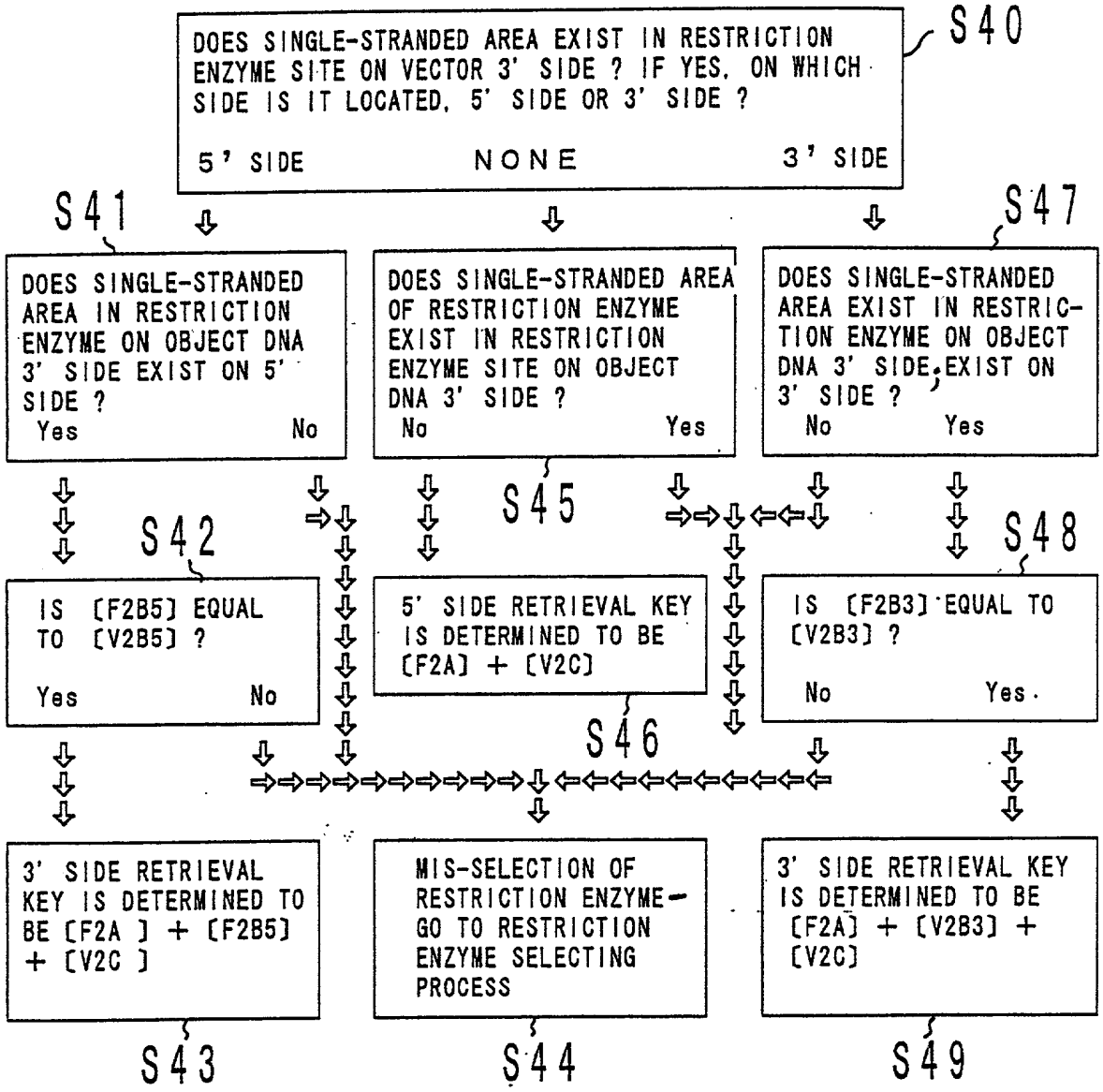

DOES SINGLE-STRANDED AREA EXIST IN RESTRICTION ENZYME SITE ON VECTOR 3' SIDE ? IF YES, ON WHICH SIDE IS IT LOCATED, 5' SIDE OR 3' SIDE ?
5' SIDE
NONE
3' SIDE
S40
S41
S47
DOES SINGLE-STRANDED AREA IN RESTRICTION ENZYME ON OBJECT DNA 3' SIDE EXIST ON 5' SIDE ?
Yes
No
DOES SINGLE-STRANDED AREA OF RESTRICTION ENZYME EXIST IN RESTRICTION ENZYME SITE ON OBJECT DNA 3' SIDE ?
No
Yes
DOES SINGLE-STRANDED AREA EXIST IN RESTRICTION ENZYME ON OBJECT DNA 3' SIDE, EXIST ON 3' SIDE ?
No
Yes
S45
S42
S48
IS [F2B5] EQUAL TO [V2B5] ?
Yes
No
5' SIDE RETRIEVAL KEY IS DETERMINED TO BE [F2A] + [V2C]
IS [F2B3] EQUAL TO [V2B3] ?
No
Yes
S46
3' SIDE RETRIEVAL KEY IS DETERMINED TO BE [F2A] + [F2B5] + [V2C]
MIS-SELECTION OF RESTRICTION ENZYME-GO TO RESTRICTION ENZYME SELECTING PROCESS
3' SIDE RETRIEVAL KEY IS DETERMINED TO BE [F2A] + [V2B3] + [V2C]
S43
S44
S49

FIG. 16

WHEN HIND III IS SPECIFIED ON VECTOR 5' SIDE XBA I IS SPECIFIED ON VECTOR 3' SIDE, HIND III IS SPECIFIED ON OBJECT DNA 5' SIDE, AND XBA I IS SPECIFIED ON OBJECT DNA 3' SIDE

(**** INDICATES RESIDUAL MULTIPLECLONING SITE
(+++++ INDICATES AN OBJECT DNA FRAGMENT

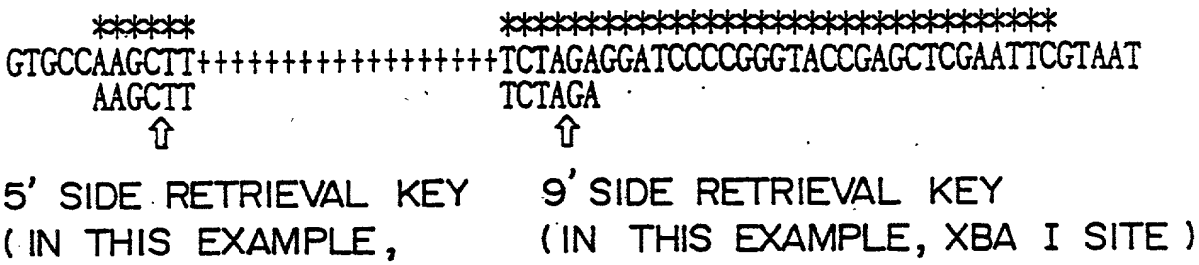


FIG. 17

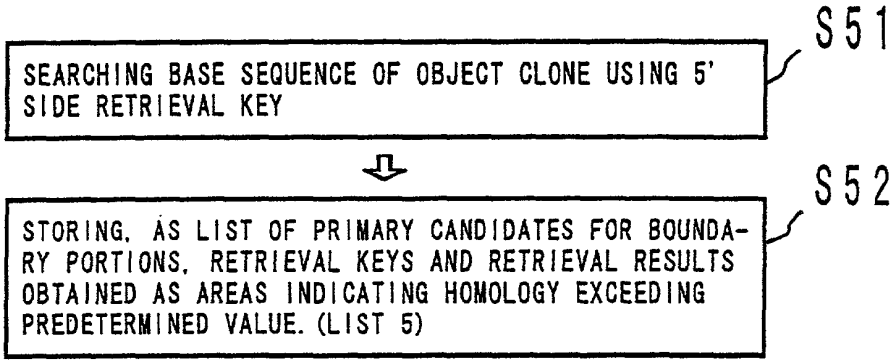

S51
SEARCHING BASE SEQUENCE OF OBJECT CLONE USING 5' SIDE RETRIEVAL KEY
S52
STORING, AS LIST OF PRIMARY CANDIDATES FOR BOUNDARY PORTIONS, RETRIEVAL KEYS AND RETRIEVAL RESULTS OBTAINED AS AREAS INDICATING HOMOLOGY EXCEEDING PREDETERMINED VALUE. (LIST 5)

FIG. 18

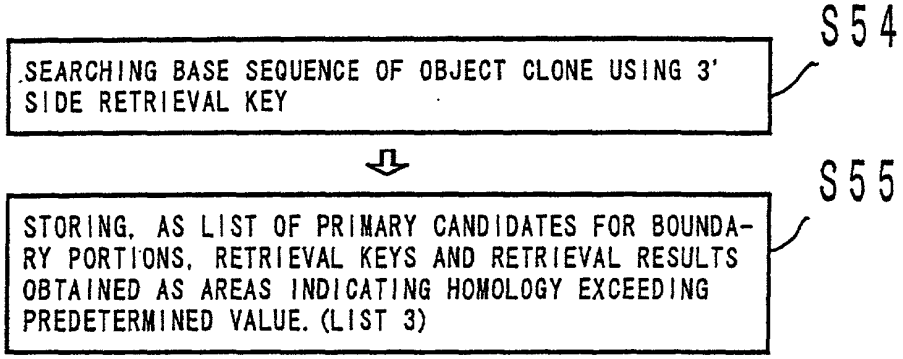


FIG. 19

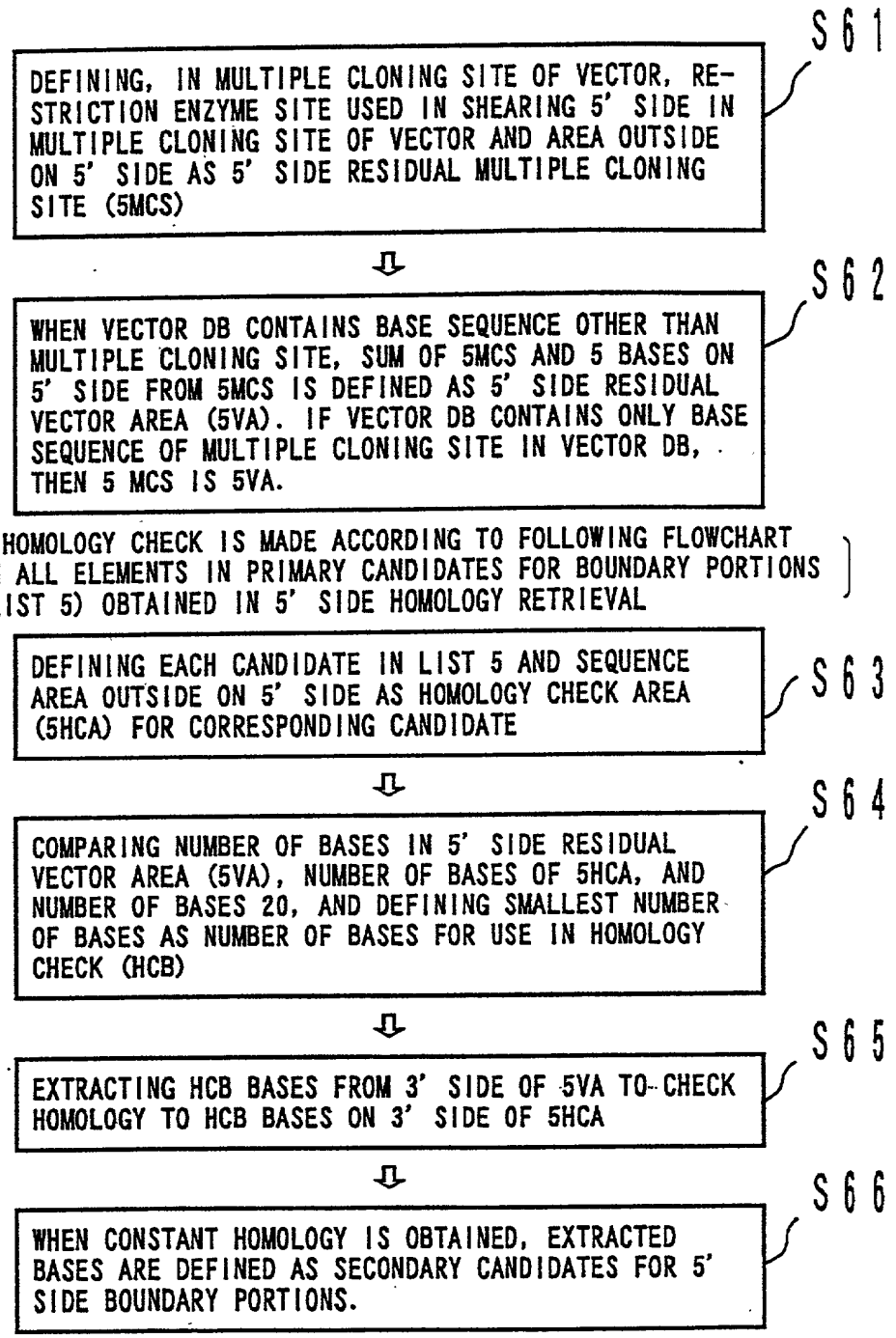


FIG. 20

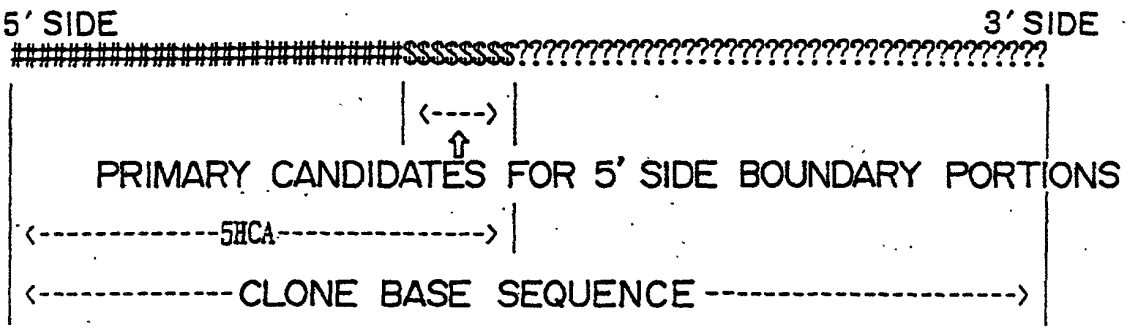


FIG. 21

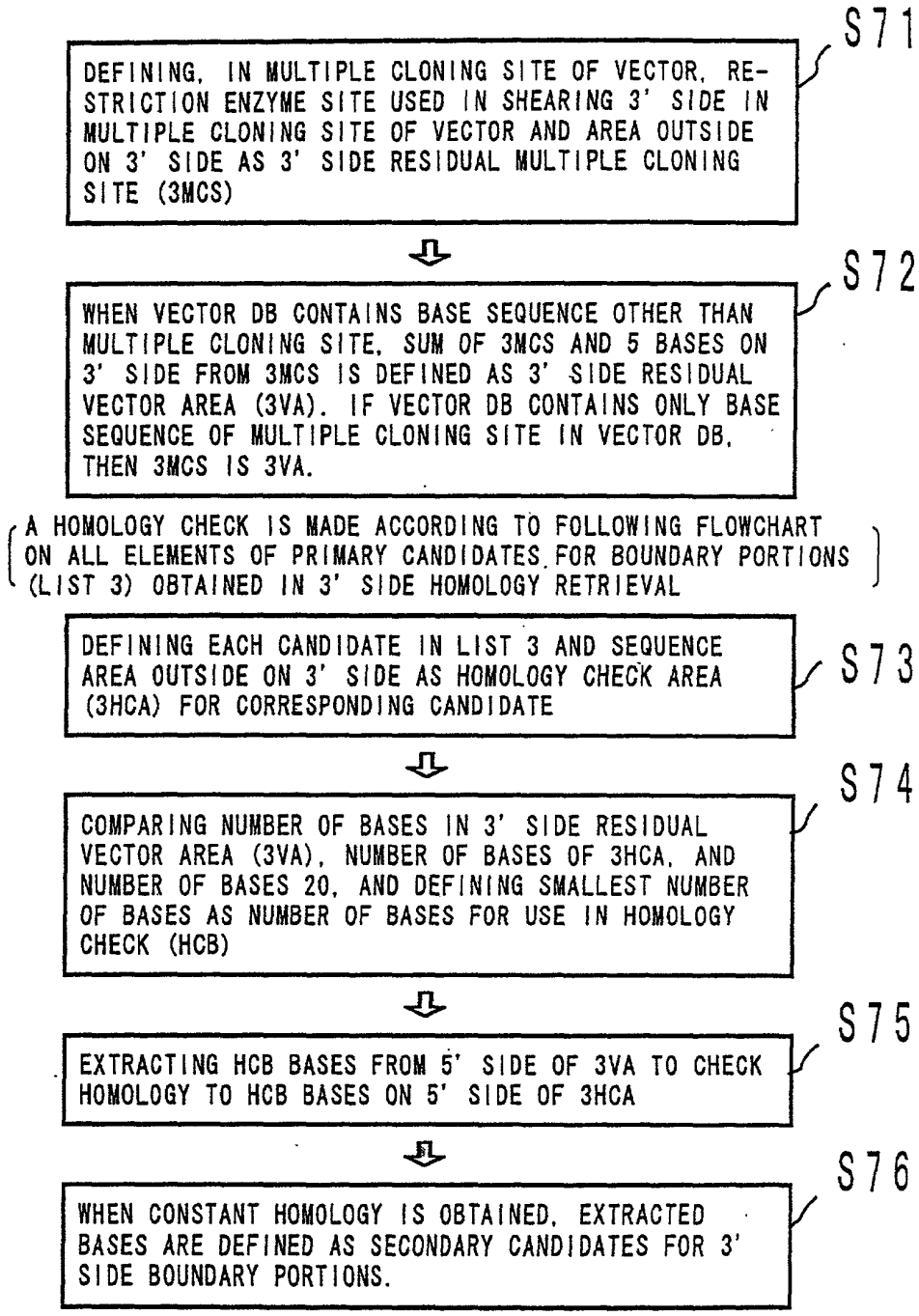


FIG. 22

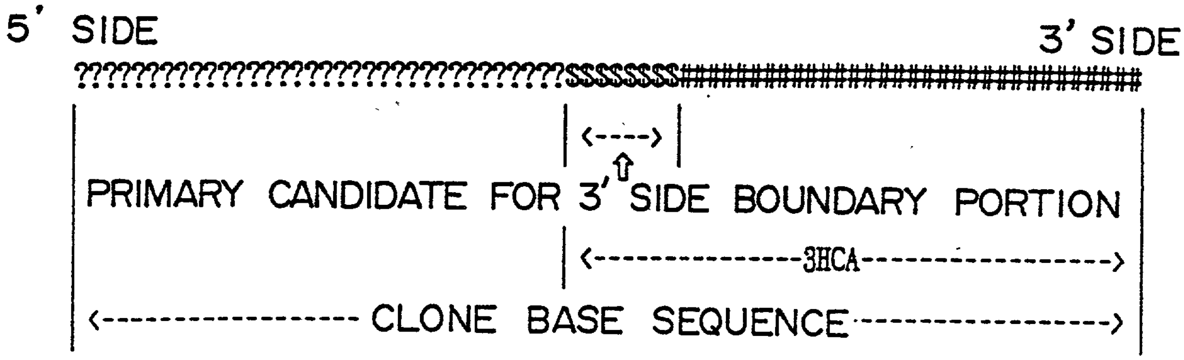

5' SIDE
3' SIDE
?????????????????????????????????SSSSSSSS################################
PRIMARY CANDIDATE FOR 3' SIDE BOUNDARY PORTION
3HCA
CLONE BASE SEQUENCE

FIG. 23

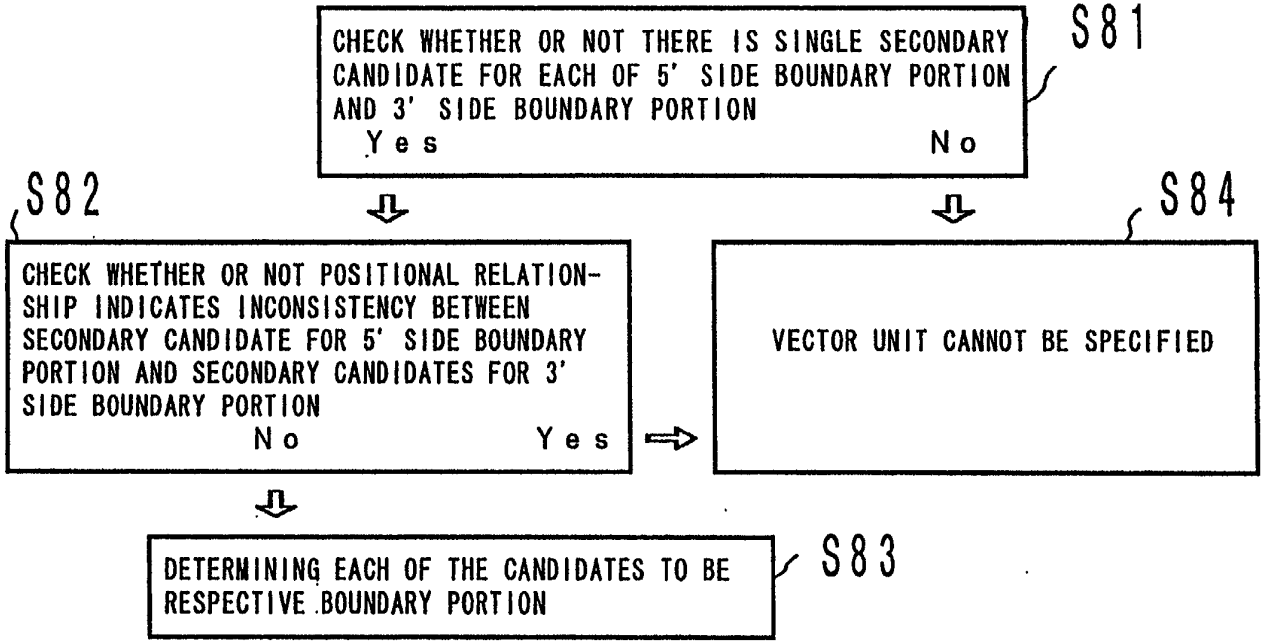

CHECK WHETHER OR NOT THERE IS SINGLE SECONDARY CANDIDATE FOR EACH OF 5' SIDE BOUNDARY PORTION AND 3' SIDE BOUNDARY PORTION
Yes
No
S81
S82
CHECK WHETHER OR NOT POSITIONAL RELATION-SHIP INDICATES INCONSISTENCY BETWEEN SECONDARY CANDIDATE FOR 5' SIDE BOUNDARY PORTION AND SECONDARY CANDIDATES FOR 3' SIDE BOUNDARY PORTION
No
Yes
S84
VECTOR UNIT CANNOT BE SPECIFIED
DETERMINING EACH OF THE CANDIDATES TO BE RESPECTIVE BOUNDARY PORTION
S83

FIG. 24